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Pathogens' Exploitation of the Intestinal Food Web

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Competition for nutrients is a key factor controlling pathogen colonization within the gastrointestinal tract. In this issue, Ferreyra et al. (2014) and Curtis et al. (2014) show that diverse enteric pathogens can exploit a metabolic byproduct from the commensal microbiota, succinate, to enhance their own virulence expression and proliferation.

The human large intestine is home to a complex and dynamic consortium of microbes, also known as the microbiota. Akin to a physiologic organ, the microbiota fulfils many important functions to maintain host health, including nutrition acquisition, immune development, and pathogen defense. Among the most well-studied functions of the microbiota is its tremendous metabolic capacity. The microbiota contributes to the production of vitamins (e.g., vitamins K and B12 and folic acid), enhances ionic absorption of calcium and magnesium, and is indispensable for energy salvage from nutrients otherwise nondigestible by human cells (Gill et al., 2006). Interestingly, while some metabolic activities of the microbiota such as methanogenesis or oxalate degradation may vary widely between different individuals, the ability to ferment nondigestible carbohydrates and proteins is highly conserved among those functions encoded by the human core microbiome (Qin et al., 2010).

The principle end products of bacterial metabolism in the large intestine are short-chain fatty acids (SCFAs), which mainly arise from anaerobic fermentation of nondigestible carbohydrates. In the large bowel, these consists of nonstarch polysaccharides (e.g., dietary fiber and plant polysaccharides including cellulose and pectin), resistant starch, simple carbohydrates not yet digested in the small intestine, as well as those derived from sloughed goblet cells and host mucin glycoproteins. As small (1- to 6-carbon) organic fatty acids, SCFAs are readily absorbed and metabolized by the intestinal mucosa and the liver, where they play a substantial role in host nutrition (Macfarlane and Macfarlane, 2003). SCFAs can also modulate the intestinal immune response, such as by inhibiting excessive proinflammatory cytokines in chronic inflammation (Smith et al., 2013), and exert a direct trophic effect on the intestinal epithelium. While the beneficial effect of microbiota-associated metabolites, particularly SCFAs, to the host has been a subject of intense research, how these substrates influence bacterial-bacterial interactions is less well understood.

The metabolic landscape of the gut can vary greatly depending on the microbiota structure, intestinal transit time, and availability of fermentable substrates. However, butyrate (C4), propionate (C3), and acetate (C2) are typically the most abundant and together make up over 80% of total SCFAs produced during health (Macfarlane and Macfarlane, 2003). These molecules generally arise from oxidative metabolism of hexose sugars (e.g., glucose and fructose) through the nearly ubiquitous Embden-Meyerhof glycolysis pathway. Other SCFAs such as valerate, formate, and isobutyrate are products of branched-chain amino acid metabolism and exist in much lower levels, while lactate, ethanol, and succinate are metabolic intermediates that are converted to other SCFAs to various degrees depending on the extent of microbial cross-feeding within the intestinal microenvironment. Therefore, these intermediates are rarely present at significant levels in the gut. Interestingly, succinate has been found to accumulate during intestinal inflammation, such as in antibiotic-associated diarrhea and infection with the pathogen *Clostridium difficile* in human and mice (Lawley et al., 2012), although its role in disease pathogenesis is still unclear.

In this issue of *Cell Host & Microbe*, two studies by Ferreyra et al. (2014) and Curtis et al. (2014) present important mechanistic insights into the role of succinate in commensal-pathogen interactions within the competitive gut ecosystem. Importantly, both studies provide compelling evidence to suggest that intestinal metabolites induced by a commensal bacterium, *Bacteroides thetaiotaomicron*, can unexpectedly enhance pathogen virulence expression and colonization in the gut, through the production of succinate

(Figure 1). *B. thetaiotaomicron* is a Gram-negative obligate anaerobe, which possesses an arsenal of enzymes to degrade complex polysaccharides. Among other *Bacteroides* species, *B. thetaiotaomicron* can readily produce SCFAs as well as convert host mucin into succinate and acetate, which in turn allows the production of butyrate by other secondary fermentative microbes (Fischbach and Sonnenburg, 2011). Thus, *B. thetaiotaomicron* exemplifies a commensal symbiont well-adapted to survive within and to support the intestinal metabolic network.

Curtis et al. (2014) observed that in the presence of *B. thetaiotaomicron*, the human pathogen enterohemorrhagic *E. coli* dramatically upregulates the expression of numerous virulence factors, including those encoded by the locus of enterocyte effacement (LEE) pathogenicity island. Interestingly, this transcriptional regulation is driven by a sugar-sensing mechanism, controlled by the catabolite repressor/activator protein Cra (Njoroge et al., 2012). Under nutrient-rich conditions where glycolytic catabolites (e.g., glucose and fructose) are plentiful, Cra is inhibited from binding to and activating the transcription of ler, the master regulatory gene of the LEE. In glucose-poor environments, however, *E. coli* can switch to utilizing gluconeogenic substrates such as lactate, succinate, and glycerate. Meanwhile in these gluconeogenic conditions, Cra can bind to the *ler* promoter and enhance expression of the LEE genes. This is thought to be an important virulence mechanism for *E. coli* to establish colonization at the intestinal mucosa and attach to the host epithelial surface, where glucose is far less abundant than in the gut lumen. Thus, the finding by Curtis et al. (2014) indicates that sensing of metabolites produced by the commensal *B. thetaiotaomicron* can direct virulence and colonization potential of a pathogen.

Using the *Citrobacter rodentium* infection model in mice, which mimics human *E. coli* infection, the authors further identified succinate as a key metabolite during pathogenesis in vivo. In the presence of *B. thetaiotaomicron*, succinate level rises significantly during *C. rodentium* infection. This in turn enhances the expression of *C. rodentium* virulence genes such as *ler* and *eae*, responsible for the formation of attaching-effacing lesions on epithelial cells that are central to *C. rodentium* pathogenicity. Accordingly, mice infected with *C. rodentium* experienced a greater morbidity and more severe intestinal pathology when their microbiota had first been colonized by *B. thetaiotaomicron*. Moreover, this effect was not associated with a higher density of *C. rodentium* in the gut, but rather an increase in its virulence and a greater loss of various protective mucosal mechanisms such as the production of mucin and antimicrobial peptides.

Ferreyra et al. (2014) studied the major metabolic pathways employed by another intestinal pathogen, *Clostridium difficile*, in the presence of *B. thetaiotaomicron*. *C. difficile* infection is an important cause of antibiotic-associated diarrhea worldwide and typically occurs when the indigenous microbiota is severe disturbed by antibiotic exposure (Lawley et al., 2012). In this study, the authors used a simplified gnotobiotic mouse model, in which mice were either monocolonized with each bacterial species or both *C. difficile* and *B. thetaiotaomicron*. In addition, mice were fed with a normal (polysaccharide-rich) diet or a polysaccharide-deficient diet in order to shift the metabolic landscape of the gut. Similarly to the observations made in *E. coli*, Ferreyra et al. (2014) noted that *C. difficile* was able to respond rapidly to the availability of carbohydrates in the gut and the presence of *B. thetaiotaomicron* by changing its own metabolic programs. Consistent with a previous report by Ng et al. (2013), cocolonization with *B. thetaiotaomicron* under polysaccharide-poor condition led to enhanced metabolism of sialic acid by *C. difficile*. Sialic acids are monosaccharides naturally occurring at the end of host mucin molecules and epithelial cell surfaces that can be released enzymatically by *Bacteroides* spp. Thus, *C. difficile* has evolved a strategy to utilize a nutrient derived from host-commensal interactions to enhance its own colonization.

Furthermore, when polysaccharides are abundant and in the presence of competition by *B. thetaiotaomicron*, *C. difficile* switches on alternative metabolic pathways allowing the utilization of sorbitol and fructose and the conversion of *Bacteroides*-produced succinate to butyrate. Importantly, induction of the succinate-to-butyrate pathway also promotes proliferation and host colonization by *C. difficile*. This is demonstrated by the observation that given an abundance of succinate in the gut, a *C. difficile* mutant lacking the succinate transporter could not colonize to similarly high density as wild-type *C. difficile*. Interestingly, in addition to *C. difficile*-induced diarrhea, Ferreyra et al. (2014) found that succinate also accumulates during antibiotic treatment and during polyethylene-glycol-induced osmotic diarrhea. Thus, succinate utilization may represent a common beneficial strategy for pathogens to expand in a competitive gut ecosystem during different perturbations to the microbiota.

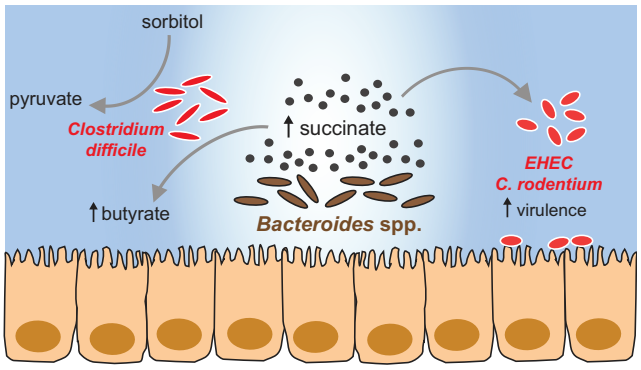

Figure 1. Interplay between Commensal-Derived Metabolites and Pathogen Colonization

The intestinal metabolic environment is typically dominated by the common SCFAs butyrate, propionate, and acetate. Succinate is a major product excreted by Bacteroides spp., but it is typically consumed by secondary fermentative microbes and therefore rarely accumulates to appreciable level, unless during inflammatory conditions or antibiotic treatment. During infection, *C. difficile* can couple the conversion of succinate to butyrate with the fermentation of dietary carbohydrates such as sorbitol, thereby enhancing its growth and colonization. At the same time, succinate promotes gluconeogenetic metabolism in enterohemorrhagic *E. coli* (EHEC) and *C. rodentium*. This can drive expression of their virulence genes, including those involved in the formation of attaching-effacing lesions. Thus, phylogenetically diverse enteric pathogens can subvert a commensal-derived metabolite for colonization and virulence.

Intestinal pathogens are well known to exploit the host inflammatory response or host-derived metabolic byproducts to outcompete other microbes and establish colonization. Here, Ferreyra et al. (2014) and Curtis et al. (2014) both found that a commensal-derived metabolite, succinate, can also directly benefit diverse pathogens belonging to the Firmicutes (*C. difficile*) and the Proteobacteria (EHEC and *C. rodentium*) phyla (Figure 1). Thus, although the commensal microbiota often plays a fundamental role in colonization resistance against invading pathogens, enteric pathogens also have evolved different strategies to exploit metabolic intermediates available in the inflamed gut for their own advantage (Keeney and Finlay, 2011). Together, this work represents valuable insight into the function of microbiota-derived metabolites, which can guide the development of approaches to attenuate pathogen virulence or colonization by modulating the intestinal metabolic pathways.

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Using Fat to Turbo-Charge Intracellular Parasite Growth

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Early during infection, the malaria parasite invades liver cells and undergoes robust replication, generating thousands of new parasites within days. In this issue of *Cell Host & Microbe*, Itoe et al. (2014) show that parasite replication in the liver depends on the synthesis of host bulk phospholipids, which are incorporated into the expanding parasite and surrounding vacuolar membranes.

Many microbial pathogens have evolved strategies for surviving and replicating within host cells. While an intracellular niche offers some sanctuary from the host immune system, intracellular pathogens face the challenge of scavenging all of their essential nutrients and carbon sources from the host cell. Many pathogens achieve balanced growth within their intracellular niche by greatly reducing their overall growth rate. However, a few pathogens have evolved strategies for commandeering host nutrients and metabolic precursors in a way that allows high growth rates within their respective host cells. A spectacular example of this is the liver stage of the malaria parasite (Prudêncio et al., 2006). Infection of the liver by this parasite occurs immediately after injection of *Plasmodium* sporozoite stages into the skin of the mammalian host by the mosquito vector and is an obligate step in the development of clinical infection. Following invasion of a liver hepatocyte, the parasite is surrounded by a vacuolar membrane, generating an intracellular compartment in which the parasite undergoes a burst of nuclear replication and daughter cell formation. In the case of the human malaria parasite, *Plasmodium falciparum*, infection of a single hepatocyte gives rise to 10,000–30,000 new parasites (merozoites) over 5 to 6 days. These parasite progeny are released from their vacuolar compartment and finally from their host cell to initiate cycles of infection in red blood cells leading to the symptoms associated with malaria. The rate of *P. falciparum* replication in hepatocytes is almost without precedent in eukaryotic biology and greatly exceeds the replication of bloodstream parasite stages. There is obvious interest in understanding how intrahepatic *Plasmodium* stages achieve